CASE REPORT: MANTLE CELL LYMPHOMA WITH ABNORMAL CD117 COEXPRESSION

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Mantle cell lymphoma (MCL) is a neoplasm of mature lymphocytes commited to the B-cell lineage. This neoplasm has an aggressive clinical course, with poor response to standard treatment and short overall survival. MCL typical immunophenotype has been described as a mature monoclonal B-cell population with expression of CD19, CD20, CD43, CD45, CD79 and CD5; and negative expression of CD10, CD23 and CD200. Overexpression of cyclin D1 as detected by immunohistochemistry is characteristic of this disease. CD117 or c-kit is a surface protein encoded by the proto-oncogene *c-kit* which is expressed in normal hematopoietic and neoplastic cells, usually of myeloid lineage. There are few published studies with assessment of CD117 expression on mature B-cell neoplasms (MBCN). In this report, we described a MCL case with aberrant CD117 coexpression as demonstrated by flow cytometry immunophenotyping (FCI). Patient was a 64 year-old male with leukocytosis (39.20 x 10^9/l) and lymphopenia with normal red blood cell and platelet counts. Peripheral blood FCI with a screening panel evidenced an abnormal population representing 64% of nucleated cells, with expression of CD19, CD45(bright) and CD117. Additional staining of myeloid and lymphoid markers was performed. The neoplastic cells had positive expression of cytCD79a, CD5, CD19, CD20(bright), CD27, CD38, CD45(bright), CD43, CD79b, CD81, HLA-DR and kappa light chain; and were negative for cytCD3, cytMPO, CD10, CD11c, CD23, CD25, CD33, CD34, CD103, CD200 and lambda light chain. CD117 PE (clone 104D2, EXBIO, Praha, CZE) staining was repeated and positive expression was confirmed. FCI findings were compatible with the diagnosis of MCL with aberrant CD117 coexpression. After initial chemotherapy, patient had persistent leukocytosis (19.10 x 10^9/l) with anemia (hemoglobin of 11.1 g/l). Bone marrow aspirate FCI showed persistence of 37% of neoplastic cells. Unfortunately, patient died from causes unrelated to the disease before the next chemotherapy cycle. CD117 expression is a common finding in acute myeloid leukemia, being less frequently expressed in precursor lymphoid neoplasms and rarely identified in mature neoplasms. There are few studies evaluating CD117 expression in MBCN since its assessment is not part of the diagnostic panels. Assessment of *c-kit* mRNA expression in different types of lymphomas only identified its expression in CD30-positive cases. A CD117-positive B-cell non-Hodgkin lymphoma (NHL) carrying the t(14;18) translocation has been described in a case report. In another study, CD117 overexpression was identified in 2 out of 17 MCL cases using immunohistochemistry. Also, CD117 expression was identified in four out of 30 diffuse large B-cell lymphoma (DLBCL) cases. There is one large study assessing the distribution and expression of KIT in 824 cases of malignant lymphoma where CD117 expression was assessed with tissue microarray, and only one B-cell follicular lymphoma had positive CD117 expression. The role of CD117 expression on MBCN is still unclear, however, the abnormal expression of this marker has potential to be used for FCI minimal residual disease (MRD) assessment. In conclusion, CD117 expression can be found in MCBN such as MCL and expansion of FCI panel in order to better characterize such abnormal populations is necessary for the correct diagnosis.

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CD19-NEGATIVE B-LYMPHOBLASTIC LEUKEMIA/LYMPHOMA: A CASE REPORT

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Lymphoblasts in B-lymphoblastic leukemia/lymphoma are almost always positive for the B-cell markers CD19, cytCD79a and cytCD22 and, according to the revised 4th edition of the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues, expression of those markers in combination or at high intensity supports B-cell lineage assignment. We had a case of CD19-negative B-lymphoblastic leukemia/lymphoma, which represented a diagnostic challenge. Patient was a 20 year-old female with clinical suspicious of acute leukemia. At physical examination she had splenomegaly. Complete blood cell counts at admission showed hemoglobin of 4.9 g/dl, white blood cell of 5.1 $\times 10^9$/l (neutrophils – 26%; lymphocytes – 72%) and platelet count of 36 $\times 10^9$/l. Lactate dehydrogenase levels were increased. Flow cytometry immunophenotyping (FCI) assessment of a bone marrow sample revealed an immature population that expressed cytCD22, CD10^{bright}, CD22, CD34, CD38, CD45^{weak}, CD99, HLA-DR and nuTdT, and coexpressed CD123. Blast cells were negative for cytCD3, cytCD79a, cytIgM, cytMPO, CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD33, CD36, CD41, CD56, CD58, CD79a, CD71, CD81 and CD117. Assessment of CD19 expression was performed with CD19 PerCP (clone 4G7, from EXBIO, Praha, CZE) and CD19 APC (clone HIT2, from BD Biosciences, San Diego, USA). Assessment of peripheral blood and bone marrow smears were performed elsewhere and karyotype results were not available at the time of diagnosis. Patient's final FCI diagnosis was of CD19-negative B-lymphoblastic leukemia/lymphoma. Although CD19 is a reliable surface marker for B-cells, it is not expressed in a minor subpopulation of precursor B-cells throughout normal lymphopoiesis, such subset can be identified by CD22 expression. During B-cell ontogeny, CD19 surface expression can be detected around the time of immunoglobulin gene

rearrangement, which explains the absence of cytoplasmic IgM in our case. There are few published cases of CD19-negative B-lymphoblastic leukemia/lymphoma and the precise diagnosis of this entity requires expanded FCI panels for the exclusion of other hematopoietic neoplasms. In conclusion, FCI has an important role in the diagnosis, classification and monitoring of hematopoietic neoplasms and it is necessary to test for the expression of all B-cell markers as recommended by the WHO for the correct assignment of B-cell lineage.

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CITOMETRIA DE FLUXO COM MARCAÇÃO EPCAM EM LÍQUIDO CEFALORRAQUIDIANO EM NEOPLASIAS NÃO HEMATOLÓGICAS

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Objetivos: Avaliar a utilização da citometria de fluxo (CF) em amostras de líquido cefalorraquidiano (LCR) como ferramenta de triagem, confirmação diagnóstica e acompanhamento de neoplasias não hematológicas, a partir da adição do marcador epitelial CD326, comparando seu desempenho com a técnica convencional citomorfológica. **Materiais e métodos:** Estudo retrospectivo com levantamento de 1050 amostras de LCR admitidas no laboratório de CF entre 2018 e 2021. Foi utilizado equipamento FACSCANTO II e o software INFINICYT. Painel utilizado: CD36, ROR1, CD38, CD10, CD56, CD326, CD71, CD81, CD45, CD138, CD8+Lambda, CD56+Kappa, CD4, CD19, CD3 +CD14, CD38, CD20, CD45. As amostras foram coletadas em meio com conservante celular. Resultados foram comparados com a citologia oncótica tradicional. **Resultados:** Foram identificados 8 casos sugestivos de infiltração liquorica por neoplasia não hematológica. Desses, 7 foram concordantes com a citologia oncótica positiva. As neoplasias foram adenocarcinomas gástricos (3 casos), pulmão (3 casos) e sítio primário indeterminado (1 caso). O outro caso foi seguido por outra instituição. **Discussão:** Até 8% dos pacientes com câncer desenvolvem metástases meníngeas, resultando em prognóstico desfavorável e menor sobrevida global. Ainda que a maioria das intervenções seja paliativa, o diagnóstico precoce e a instituição de tratamentos podem retardar a progressão da doença, prolongar a sobrevida e reduzir morbidade neurológica. Contudo, a identificação precoce pode ser desafiadora. O volume de LCR disponível é limitado, a avaliação citomorfológica depende da experiência do analisador e as células neoplásicas podem não se distinguir morfologicamente. A CF pode ser um método útil nessa detecção por necessitar de pequeno volume amostral e ser menos subjetiva. A escassez de estudos que demostram a importância e habilidade da CF em detectar neoplasias não hematológicas pode ser explicada pelo fato da maior parte dos painéis ser apenas direcionada para o diagnóstico de doenças oncohematológicas. Nesse contexto, citoqueratinas têm sido utilizadas para especificar células não hematológicas, habitualmente negativas para o CD45. O CD326 ou EpCAM (*Epithelial Cell Adhesion Molecule*) é uma delas. Esse marcador modula negativamente a adesão celular, tem efeito antiadesivo na migração celular e na promoção de metástases. Contudo, nem todas as neoplasias infiltrativas do espaço subaracnoideo são epiteliais. Exemplo é o melanoma, a terceira maior causa de meningite carcinomatosa, na qual não há expressão de CD326. Além disso, algumas células normais epiteliais podem também expressá-lo. **Conclusão:** Em análise retrospectiva, a CF demonstrou ser um método rápido, eficaz e seguro para o diagnóstico de invasão de sistema nervoso central por neoplasia não hematológica. Apresenta reprodutibilidade, custo-efetividade e sensibilidade, desde que as amostras de liquor sejam preservadas adequadamente no transporte e armazenamento. Sugere-se ampliação da inclusão do marcador CD326 na rotina de análise oncótica de LCR, quando houver hipótese clínica de infiltração por neoplasia não hematológica.

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DETECTION OF HEMATOPOIETIC NEOPLASMS IN CEREBROSPINAL FLUID BY IMMUNOPHENOTYPING: SINGLE-CENTER DATA ANALYSIS

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Introduction: Cerebrospinal fluid (CSF) infiltration can be identified at diagnosis and during the course of many hematopoietic malignancies, mainly acute leukemia and aggressive non-Hodgking lymphomas (NHL). The accurate diagnosis of central nervous system (CNS) infiltration allows patients with infiltrated CSF to be properly treated, avoiding the use of unnecessary CNS therapy for patients who do not have compromised CSF. Cytomorphology is the classic method for diagnosing CSF neoplastic infiltration, but it presents variability in sensitivity and specificity according to the experience of the morphologist and cell abnormalities resulting from sample preparation. Currently, multiparametric flow cytometry (MFC) has great sensitivity and specificity to detect cells of hematopoietic neoplasms in the CSF. This is a retrospective study to assess the frequency of CSF infiltration in a cohort of patients with hematologic malignancies treated at our institution. **Aim:** to evaluate the casuistry and frequency of CSF infiltration by hematologic neoplasms diagnosed by immunophenotyping in our institution. **Material and methods:** the results of CSF immunophenotyping were collected using the flow cytometry laboratory database. Assess period: from March 2008 to June 2021. Patients were grouped by type of disease and the frequency with which each one was represented in the cohort was verified. **Results:** a total of 1572 CSF samples were analyzed by the laboratory during the study period. The frequency of diagnoses of patients whose samples were sent for evaluation were, in decreasing order: 956(60.8%), B-cell precursor acute